

BRCA1/BARD1 mediate apoptotic resistance but not longevity upon mitochondrial stress in *C. elegans*

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision

5 April 2018

Thank you for the submission of your research manuscript to our journal. I apologize for the delay in handling your manuscript, but we have only recently received the last referee report and I also discussed the reports further with the referees. Please find the full set of reports copied below.

As you will see, the referees acknowledge that the findings are potentially interesting but all referees also raise a number of concerns and have a number of suggestions for how the study should be strengthened. It appears that one of the major concerns here is that the numbers of mitotic and meiotic nuclei are already reduced at baseline in *frh-1* or *isp-1* deficient worms. It will thus be essential to normalize the observed changes in apoptosis and proliferation upon IR-induced DNA damage to the total number of cells. Moreover, it should be tested if the mutants are desensitized or if they can still respond to very high radiation doses.

In addition, referee 1 suggested to reduce mitochondrial function specifically in the soma and the germline to distinguish systemic from germline-specific effects. Referee 2 further states that no direct measurements of increased repair or genome maintenance in the MTC mutants are provided. Upon further discussion with the referees, we suggest to address these issues with a more careful description of the results and by toning down the respective conclusions.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient

for the revisions so that we can discuss the revisions further.

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied. Please also include scale bars in all microscopy images.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (<http://embor.embopress.org/authorguide#revision>). Please insert page numbers in the checklist to indicate where the requested information can be found.
 - a letter detailing your responses to the referee comments in Word format (.doc)
 - a Microsoft Word file (.doc) of the revised manuscript text
 - editable TIFF or EPS-formatted figure files in high resolution
- (In order to avoid delays later in the publication process please check our figure guidelines before preparing the figures for your manuscript:
http://www.embopress.org/sites/default/files/EMBOPress_Figure_Guidelines_061115.pdf)
- a separate PDF file of any Supplementary information (in its final format)
 - all corresponding authors are required to provide an ORCID ID for their name. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<http://embor.embopress.org/authorguide>).

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I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFeree REPORTS

Referee #1:

The manuscript by Torgovnick et al. probes the relationship between DNA damage and lifespan through analysis of worms with reduced mitochondrial (mit) function. They conclude that these processes can be uncoupled as exemplified by inactivation of the tumor suppressor BRC-1/BRD-1 and analysis of DNA damage response and lifespan. This is a well written study of interest to a wide group of biologists. However, the manuscript would be strengthened by some reorganization and further analyses.

Figures 1 and 2. Given the reduction in the numbers of both mitotic and meiotic germ cell nuclei in worms with reduced mitochondrial function (*frh-1* and *isp-1*) (Fig 1A and B), I recommend that the data on H3S10P, CDK1, EdU and apoptosis, also be expressed as a ratio to normalize the number to total germ cell, as the overall ratios of H3S10P, EdU and apoptotic positive cells may be similar in wild type and under mitochondrial stress. I would also like to see the statistics of +/- IR upon *frh-1* and *isp-1* depletion. If there is no statistical difference, please state. Note in supplementary figure 1B and C, the images do not show the distal germ line, as indicated in the figure legend.

On figure S2 E please label as *ced-9* directly on the graph.

In figure 3, data on transcriptional activation of *egl-1* and *ced-13* is shown in the mit mutants in response to IR and UVB as a measure of the apoptotic signaling pathway. The authors then show PARP and RPA staining in the proliferative zone. I recommend that the authors be more careful in distinguishing the consequence of DNA damage in activating apoptosis at the bend of the gonad in late meiotic prophase versus cell cycle arrest in the proliferative zone. Perhaps the PARP and RPA staining can be included in Figure 4 where the authors show specific data of cell cycle arrest? Again, I recommend that the PARP and RPA data be presented as per nuclei to account for differences in numbers - in this case it is likely to accentuate the differences. The consequence of DNA damage to embryonic lethality upon reduction of *frh-1* and *isp-1* should be shown in addition to the brood size (Fig 4E-G).

Throughout the manuscript the authors distinguish between germline function and systemic function, yet all of the experiments are performed on worms where mitochondrial function is reduced systemically. The authors should examine the phenotype of reducing mitochondrial function specifically in the germ line as well as specifically in the soma. This would enable them to really distinguish a systemic effect from a germ line effect and may provide insight into why lifespan is uncoupled from DNA damage response under some conditions.

Referee #2:

In this manuscript by Torgovnick et al, the authors investigated whether lifespan extension in *C. elegans* by reducing mitochondrial function through knockdown of *frh-1* and *isp-1* causally correlates with resistance to genotoxic stress. The authors first show that *frh-1* and *isp-1* knockdown leads to reduced germ cell proliferation and apoptosis and absence of DNA damage induced cell cycle arrest and apoptosis. As the apoptotic machinery appears intact, the authors conclude that reducing mitochondrial function promotes DNA damage avoidance or detoxification/repair. To identify genes that mediate this 'protective response', the authors perform an RNAi screen and identify *brc-1* and *brd-1* as (partial) mediators of this response. Finally, lifespan experiments are performed, which suggest that the protective effect in the germline is not linked to somatic lifespan extension.

This manuscript reports a novel finding that will be of interest to a broad readership. However, my main concern is that the conclusion of the authors (in the abstract and throughout the manuscript) that there is a protective 'effect' after *frh-1* and *isp-1* knockdown due to 'a more efficient genomic maintenance apparatus' is not convincingly substantiated by the experimental evidence. It is nowhere shown that there is indeed increased DNA maintenance or repair, by means of direct measurement of DNA damage removal or (germ cell) survival. The authors only show that in the

absence of *isp-1* or *frh-1* there is a lack of apoptosis induction, which is a very indirect measure of genome maintenance. Lack of apoptosis does not necessarily mean that genome maintenance is improved (see for instance Stergiou et al, Cell Death and Differentiation 2007). The authors should either show increased repair/genome maintenance or rephrase their conclusions more carefully and/or discuss other interpretations in accord with their actual data.

The idea of improved genome maintenance is striking and should therefore be firmly established, because the same 'protective' effect is observed using agents that create different types of DNA damage requiring different repair pathways for removal. Do the authors envision that these different DNA repair pathways are all enhanced? For instance, would *brc-1/brd-1* also act in response to UV damage?

On page 7, the authors conclude that DNA damage induced replication arrest is 'significantly' overcome in animals depleted of *isp-1* or *frh-1*. However, the levels of mitotic nuclei in *isp-1* and *frh-1* depleted animals are already as low as in control animals after ionizing radiation. If there already is an arrest, how can the authors then conclude that, if no further reduction in mitotic levels is observed, the arrest is 'significantly' overcome? Would a further reduction be even possible? This should be clarified.

It is unclear what is shown in Figure 4C-D. Is this after ionizing radiation (not stated in the legend)? The authors write that 'The ability to bypass a transient G2/M arrest in the germline of mitochondrial perturbed animals is also reflected by the rescuing effects on p-histone-H3 and CDK-1 expression upon IR-induced DNA-damage (Figure 4C-D, compare to panels 1C-D).' However, comparing Figure 4C-D (assuming this is after IR) to Figure 1 C-D shows that in control animals there are less PH3 positive cells after ionizing radiation, but this is already the situation for unperturbed animals with depletion of *frh-1* or *isp-1*. There is no rescue of an effect. Besides, the amount of CDK-1 positive cells seems to increase in control animals as well as *frh-1* and *isp-1* animals after ionizing radiation. This should be clarified. The authors should perform these experiments (with and without ionizing radiation) simultaneously in the same experiment and show results in the same graph so that it will be possible to properly compare results and determine significance of any differences.

How can the authors be sure that the observed reduced apoptosis after *frh-1* and *isp-1* knockdown, even after genotoxic stress, is not (partially) related to the fact that there are less pachytene cells, also because proliferation in the distal gonad is reduced?

On page 6, it is written 'upon *frh-1* and *isp-1* RNAi the number of apoptotic cells did not increase at any observed time point neither under physiological conditions nor in response to DNA-damage (Figure 2D-E).' However, Figure 2D- E clearly shows an increase in the number of apoptotic cells in time in both *isp-1* and *frh-1* knockdown animals. This is suggestive of delayed apoptotic response, possibly also because proliferation and progression through meiotic stages is slower in these animals. The authors should address this issue. Also, the authors should clarify whether in *frh-1* and *isp-1* knockdown animals more than 12 h after the high dose of 1200 J/m² of UV-B irradiation there is still any progression of cells through meiosis at all? Could it be that meiotic maturation or progression is arrested and therefore apoptosis cannot take place anymore?

Minor comments:

On page 6, the authors refer to Figure S2E when writing that in *ced-9* loss of function mutants with knockdown of *isp-1* and *frh-1* there is rescued apoptosis also after DNA-damage. This is however not shown in the figure. Please clarify.

The authors should speculate why there is less PARP1 and an increase in RPA foci formation in response to ionizing radiation when *frh-1* or *isp-1* are knocked down, which is now not discussed and therefore unclear. Could the lack of PARP1 staining and increased ionizing radiation induced RPA foci indicate that (against the main conclusion) DNA repair is not functioning properly or that there is, somehow, persistent replication stress (and/or unrepaired DNA damage) in *frh-1* and *isp-1* depleted animals?

It would help clarity of the manuscript if the authors more clearly explain their rationale for testing *gst-4:gfp* expression, as well as viability, development and fertility in their small RNAi screen

described on page 8. Why are they looking for RNAi clones that increase *gst-4* expression? For instance, only half way page 8 it is explained that *gst-4* expression is used as an indirect readout for the activation of compensatory damage detoxification pathways, but this should be explained earlier to make better sense. Possibly, such 'compensatory damage detoxification pathways' and GST-4 function could be explained as part of the introduction.

Table S1. It is unclear what is shown in this table. Which RNAi clones lead to increased *gst-4* expression in *isp-1* animals (only)? And where are the results for the other phenotypes scored? For instance, at the end of page 9, genes are mentioned that affect *isp-1* mutant fertility, but it is unclear where these genes are derived from. This table should be adjusted and/or better explained.

Page 8. It is unclear how the authors go from their screening results (Table S1) to *brc-1* and *brd-1*. From which exact data in the screening results is it clear that the focus should be on these two genes?

From the experiments shown in Fig 5C and D, the authors conclude that loss of *brc-1* or *brd-1* restores the ability of *frh-1* and *isp-1* depleted animals to induce apoptosis after ionizing radiation. However, an increase in ionizing radiation induced apoptosis is also observed in WT animals (contrasting results shown in Figure 2A). To be able properly compare these results, these experiments should be performed simultaneously and shown in the same graph.

Referee #3:

Summary

In this manuscript, the authors seek to explore the impact of mitochondrial stress-induced longevity on other aspects of cellular biology. Specifically, they investigate if mild stress on the mitochondrial respiratory chain (MRC) impacts cellular resistance to genotoxic stress. They demonstrate that MRC stress can induce germline resistance to genotoxic stress via reduced apoptosis and DNA-damage induced arrest. Using mit mutants in an RNAi screen, they were able to identify *brc-1* and *brd-1* (the BRCA1 and BARD1 homologs) as critical to these protective effects. Finally, the authors demonstrate that these genes, while important for enhanced genomic protection in the stressed MRC context, are dispensable for the enhanced longevity in these mutants. These, along with additional data presented, indicate that the DNA damage response (DDR) is uncoupled from the processes involved in longevity induced by MRC stress.

Review

This manuscript is quite well-written and clearly addresses its central narrative. The experiments used provide several lines of evidence for each point the authors present. The use of both *frh-1* and *isp-1* throughout the majority of experiments and the high agreement of both in their behavior provide a strong argument for the findings regarding MRC stress's impact on germline DNA maintenance and genotoxic stress resistance.

There are some minor grammatical errors that should be dealt with, as well as a few concerns that are listed below. However, these issues should be easy to resolve and once addressed, will make this manuscript an even stronger report. Overall, this manuscript provides an elegant insight into how MRC stress translates to other physiological stress resistances and longevity, while simultaneously teasing about these outcomes into separate pathways. Once these issues are addressed, this report will make a significant addition to the body of work that seeks to explain the role of mitochondrial stress in longevity, which has been a hot topic of debate in the aging field for several decades.

Points to Address

- 1.) In Fig 2B, only data for *frh-1* is presented. The authors should include *isp-1* data for robustness or address its absence.
- 2.) In Fig 2A, it is shown that the dose of 125 Gy does not impact the RNAi fed animals. A third, higher dose should also be included to demonstrate that the system can still respond to critical amounts of damage and is not desensitized. This will also strengthen the core assertion that MRC stress increases a protective response and does not simply ignore the genetic insults.
- 3.) For Fig 4A, the authors state that both *frh-1* and *isp-1* gamma-induced decrease is marginal, yet *frh-1* decrease is rather significant. This needs to be addressed or discussed, as it is ~25% decrease, similar to what is observed for the control.

- 4.) In Fig 6B, C, author's statements don't match the graphs. In Fig 6B, *frh-1* change does not appear significant. Figure legend should include statement of statistical analysis.
 5.) In Fig 6E, why does *glp-4* not demonstrate lifespan extension via germline ablation?

Minor Issues

- Figure 1E should be quantified
- For Fig 5, please include qRT-PCR for *bca-1* and *brd-1*.
- Fig 5A, no error bars for control. Please include.
- Fig S1C, please include arrows indicating the events that were scored for this analysis.

1st Revision - authors' response

29 July 2018

Point-by-point reply letter

As you will see, the referees acknowledge that the findings are potentially interesting but all referees also raise a number of concerns and have a number of suggestions for how the study should be strengthened. It appears that one of the major concerns here is that the numbers of mitotic and meiotic nuclei are already reduced at baseline in *frh-1* or *isp-1* deficient worms. It will thus be essential to normalize the observed changes in apoptosis and proliferation upon IR-induced DNA damage to the total number of cells.

As suggested we have now normalized all data before and after radiation to the number of mitotic or meiotic cells (as appropriate). Since the new analysis did not significantly affect the final interpretation of the results we decided for simplicity to include the new graphs in the supplementary data (see text for details).

Moreover, it should be tested if the mutants are desensitized or if they can still respond the very high radiation doses.

Different data indicate that *isp-1* and *frh-1* depleted animals are not completely desensitized but they can actually sense and respond to genotoxic insults although to a less extent than wild-type animals:

- 1) Figure 2 and 3A,B – radiation induced apoptosis can still be induced although not significantly
- 2) Figure 3C-F – a normal p53/cep-1 response is induced upon genotoxic stress
- 3) Figure 4B – increase in radiation-induced RAD51 foci indicating they can sense and respond to DNA damage
- 4) Figure 5C-H – the number of eggs laid and hatched is still partially reduced upon genotoxic stress
- 5) Figure 2D – cisplatin induces a significant amount of apoptosis
- 6) Figure EVIC – high doses of UVB arrest development of both WT and *frh-1* silenced animals
- 7) Figure EV1D-F – radiation induce apoptosis still occurs in the *ced-1* and *ced-9* loss of function mutants

In addition, referee 1 suggested to reduce mitochondrial function specifically in the soma and the germline to distinguish systemic from germline-specific effects.

This was indeed a very important point that we meant to address and we are very grateful that it was raised. We have now assessed both lifespan and radiation-induced germline apoptosis comparing the effects of systemic *isp-1* RNAi to that of soma- or germline-specific silencing. We obtained very interesting results supporting our conclusion that differential germline and systemic effects are induced upon MRC dysfunction. The new data have been included in the main text, Figure 8 and Table 1.

Referee 2 further states that no direct measurements of increased repair or genome maintenance in the MTC mutants are provided. Upon further discussion with the referees, we suggest to address these issues with a more careful description of the results and by toning down the respective conclusions.

The reviewer is right in that we did not provide any direct measurements of increased repair or genome maintenance in the MRC mutants. It is only through a combination of indirect evidence (i) the resistance to apoptosis (ii) coupled to the intact checkpoint activation; (iii) the resistance in the radiation sensitivity assay as opposed to DDR mutants; (iv) the high number of clones affecting *isp-1* mutant phenotypes in our RNAi screening and (v) the effects of *brc/brd* depletion, that we concluded that the *Mit* mutants may actually have improved genome maintenance (most likely as a result of transient low level DNA damage or replication stress). As indicated, in this revised version we toned down our conclusion and more carefully discussed the results to also incorporate additional potential explanations for our findings.

Referee #1:

The manuscript by Torgovnick et al. probes the relationship between DNA damage and lifespan through analysis of worms with reduced mitochondrial (*mit*) function. They conclude that these processes can be uncoupled as exemplified by inactivation of the tumor suppressor BRC-1/BRD-1 and analysis of DNA damage response and lifespan. This is a well written study of interest to a wide group of biologists. However, the manuscript would be strengthened by some reorganization and further analyses.

Figures 1 and 2. Given the reduction in the numbers of both mitotic and meiotic germ cell nuclei in worms with reduced mitochondrial function (*frh-1* and *isp-1*) (Fig 1A and B), I recommend that the data on H3S10P, CDK1, EdU and apoptosis, also be expressed as a ratio to normalize the number to total germ cell, as the overall ratios of H3S10P, EdU and apoptotic positive cells may be similar in wild type and under mitochondrial stress.

Thanks for pointing this out. As suggested we have now normalized all necessary data before and after radiation, to the number of mitotic or meiotic cells. Since the new analysis did not significantly affect the final interpretation of the results we decided to include the new graphs in the main figures:

- 1) Figure 1 – PH3, CDK1 and EdU positive cells normalized on number of mitotic cells; apoptotic corpses normalized on number of meiotic cells.
- 2) Figure 2 and 3 – apoptotic corpses normalized on number of meiotic cells.
- 3) Figure 4 – PARP, RPA1 and RAD51 positive cells normalized on number of mitotic cells.
- 4) Figure FIG1 and EV5 – PH3, CDK1 positive cells normalized on number of mitotic cells.

I would also like to see the statistics of +/- IR upon *frh-1* and *isp-1* depletion. If there is no statistical difference, please state.

All statistics are now clearly displayed either with asterisks (significantly different from control) or hashtag (significantly different from internal untreated control). When no marks are present it means that there is no statistically significant change. We stated this in the figure legends.

Note in supplementary figure 1B and C, the images do not show the distal germ line, as indicated in the figure legend.

Thanks for noticing this discrepancy. We fixed it accordingly.

On figure S2 E please label as *ced-9* directly on the graph.

We followed this suggestion for this and also for other panels.

In figure 3, data on transcriptional activation of *egl-1* and *ced-13* is shown in the *mit* mutants in response to IR and UVB as a measure of the apoptotic signaling pathway. The authors then show PARP and RPA staining in the proliferative zone. I recommend that the authors be more careful in distinguishing the consequence of DNA damage in activating apoptosis at the bend of the gonad in late meiotic prophase versus cell cycle arrest in the proliferative zone. Perhaps the PARP and RPA staining can be included in Figure 4 where the authors show specific data of cell cycle arrest? Again, I recommend that the PARP and RPA data be presented as per nuclei to account for differences in numbers - in this case it is likely to accentuate the differences.

Thanks a lot for the suggestion. We now rebuild the figures and, as suggested: i) we moved PARP and RPA panels in new Figure 4 where we also included RAD51 foci (Figure 4A-B); ii) we normalized the number of foci on number of mitotic nuclei and show the new analysis in Figure 4A-B.

The consequence of DNA damage to embryonic lethality upon reduction of *frh-1* and *isp-1* should be shown in addition to the brood size (Fig 4E-G).

This is a good point. Laid eggs are now in Figure 5C-E, while new data with embryonic survival are displayed in Figure 5F-H. Note that as known, UVB does not really affect embryonic survival in wild-type animals (nor does in the Mit mutants).

Throughout the manuscript the authors distinguish between germline function and systemic function, yet all of the experiments are performed on worms where mitochondrial function is reduced systemically. The authors should examine the phenotype of reducing mitochondrial function specifically in the germ line as well as specifically in the soma. This would enable them to really distinguish a systemic effect from a germ line effect and may provide insight into why lifespan is uncoupled from DNA damage response under some conditions.

We are pleased that this important point was raised since it gave us the opportunity to address it. We have now assessed lifespan and radiation-induced apoptosis comparing the effects of systemic *isp-1* RNAi to that of either soma- or germline-specific silencing. We obtained very interesting results, which support our previous conclusions (independent germline and systemic effects) and add a further level of complexity to the mitochondrial stress control of different life traits. Indeed, unexpectedly we found that:

- i) *soma specific RNAi* still protects against germline apoptosis but it is not sufficient to extend lifespan;
- ii) *germline specific RNAi* does not protect against genotoxic stress-induced apoptosis but still significantly extends lifespan (although to a reduced extent than systemic RNAi).

These new data, which have been included in the main text and new Figure 8 and in Table 1, indicate that a signal from the soma is required and sufficient for the germline anti-apoptotic effect promoted by mitochondrial stress; and that, together with previous collected data on germline less strains, both a soma and a germline cell-autonomous signals are instead required for optimal lifespan extension upon mild mitochondrial stress (as exemplified in Figure 8C-D).

Referee #2:

In this manuscript by Torgovnick et al, the authors investigated whether lifespan extension in *C. elegans* by reducing mitochondrial function through knockdown of *frh-1* and *isp-1* causally correlates with resistance to genotoxic stress. The authors first show that *frh-1* and *isp-1* knockdown leads to reduced germ cell proliferation and apoptosis and absence of DNA damage induced cell cycle arrest and apoptosis. As the apoptotic machinery appears intact, the authors conclude that reducing mitochondrial function promotes DNA damage avoidance or detoxification/repair. To identify genes that mediate this 'protective response', the authors perform an RNAi screen and identify *brc-1* and *brd-1* as (partial) mediators of this response. Finally, lifespan experiments are performed, which suggest that the protective effect in the germline is not linked to somatic lifespan extension.

This manuscript reports a novel finding that will be of interest to a broad readership. However, my main concern is that the conclusion of the authors (in the abstract and throughout the manuscript) that there is a protective 'effect' after *frh-1* and *isp-1* knockdown due to 'a more efficient genomic maintenance apparatus' is not convincingly substantiated by the experimental evidence. It is nowhere shown that there is indeed increased DNA maintenance or repair, by means of direct measurement of DNA damage removal or (germ cell) survival. The authors only show that in the absence of *isp-1* or *frh-1* there is a lack of apoptosis induction, which is a very indirect measure of genome maintenance. Lack of apoptosis does not necessarily mean that genome maintenance is improved (see for instance Stergiou et al, Cell Death and Differentiation 2007). The authors should

either show increased repair/genome maintenance or rephrase their conclusions more carefully and/or discuss other interpretations in accord with their actual data.

We definitely agree with the reviewer that “*lack of apoptosis induction does not necessarily mean that genome maintenance is improved*” especially since we did not provide any direct evidence for “*increased DNA maintenance or repair*”. Therefore, as suggested, we now rephrased our conclusions and discussed this important point by including more comprehensive explanations and citing additional papers including the one indicated by the reviewer.

In our original paper we had considered and experimentally ruled out different possibilities which could account for reduced apoptosis, namely:

- 1) Figure 3 and EV1 show apoptotic machinery and checkpoint activation are functional;
- 2) Figure 4 shows that Mit RNAi reduce to some extent radiation sensitivity compare to wild-type (as opposed to DDR mutants) suggesting intact or even improved DNA damage repair mechanisms;
- 3) EV2, RNAi screening indicate that DDR genes are required to specify *isp-1(qm150)* phenotypes;
- 4) *brc/brd* are induced upon Mit RNAi and the mutants reverts the apoptotic protection elicited by Mit RNAi against IR.

Although these are indirect evidence, these observation left us to hypothesize that Mit RNAi may prompt genome maintenance or detoxification pathways as part of a more general hormetic response to moderate mitochondrial stress. We hope that these clarifications, along with the rewriting of our manuscript will better support our conclusions.

The idea of improved genome maintenance is striking and should therefore be firmly established, because the same 'protective' effect is observed using agents that create different types of DNA damage requiring different repair pathways for removal. Do the authors envision that these different DNA repair pathways are all enhanced? For instance, would *brc-1/brd-1* also act in response to UV damage?

Thanks a lot for pointing out this very important issue, which we did not have the time to address in this manuscript. Indeed, the answer to this question would require a much more comprehensive and dedicated study, which we are planning as a follow up of this one (also based on our screening results). Nonetheless we now emphasized this point and included additional data.

Mit RNAi clearly protect against radiation-induced apoptosis but not cisplatin: we included data with *isp-1* in response to UVC and cisplatin (Figure 2D), indicating that they may prompt specific repair/detoxification pathways but not others. Interestingly, while *brc/brd* mutants suppressed basal and IR-induced apoptosis they fail to rescue the reduced number of mitotic cells (Figure 6) and only partially restore sensitivity to UVB-induced embryonic lethality (Figure EV3D). Finally, newly collected data show that at least at the selected dose, *brc/brd* are not as equally sensitive to UVB as they are to IR and in this case Mit RNAi still provide protection (Figure EV2E). These observations, together with the unexpected large number of RNAi clones affecting *isp-1(qm150)* phenotypes, support our conclusion that Mit RNAi most likely prompt different genome maintenance or detoxification pathways as part of a general hormetic response to moderate mitochondrial stress (possibly inducing transient levels of low DNA damage or replication arrest). Under this scenario, depletion of different DDR pathways may selectively affect Mit RNAi sensitivity to specific types of genotoxic stressors.

On page 7, the authors conclude that DNA damage induced replication arrest is 'significantly' overcome in animals depleted of *isp-1* or *frh-1*. However, the levels of mitotic nuclei in *isp-1* and *frh-1* depleted animals are already as low as in control animals after ionizing radiation. If there already is an arrest, how can the authors then conclude that, if no further reduction in mitotic levels is observed, the arrest is 'significantly' overcome? Would a further reduction be even possible? This should be clarified.

The reviewer is right that a further reduction of mitotic cells may not be possible. We rephrased the paragraph to actually refer to Mit RNAi resistance to radiation-induced arrest.

It is unclear what is shown in Figure 4C-D. Is this after ionizing radiation (not stated in the legend)? The authors write that 'The ability to bypass a transient G2/M arrest in the germline of mitochondrial perturbed animals is also reflected by the rescuing effects on p-histone-H3 and CDK-1 expression

upon IR-induced DNA-damage (Figure 4C-D, compare to panels 1C-D). However, comparing Figure 4C-D (assuming this is after IR) to Figure 1 C-D shows that in control animals there are less PH3 positive cells after ionizing radiation, but this is already the situation for unperturbed animals with depletion of *frh-1* or *isp-1*. There is no rescue of an effect. Besides, the amount of CDK-1 positive cells seems to increase in control animals as well as *frh-1* and *isp-1* animals after ionizing radiation. This should be clarified. The authors should perform these experiments (with and without ionizing radiation) simultaneously in the same experiment and show results in the same graph so that it will be possible to properly compare results and determine significance of any differences.

The two experiments shown in original Figure 1C,D and 4C,D were indeed carried out at the same time and only showed separately to fit in the specific session. As suggested we have now put the panels together in Figure EV5 and show fold changes in Figure 5 and normalized values in Figure EV5D-E. Moreover, as indicated by the reviewer, we now rephrased the paragraph to better reflect a possible reduced germ cell arrest in *frh-1*- and *isp-1*-depleted animals following radiation, rather than a rescuing effect.

How can the authors be sure that the observed reduced apoptosis after *frh-1* and *isp-1* knockdown, even after genotoxic stress, is not (partially) related to the fact that there are less pachytene cells, also because proliferation in the distal gonad is reduced?

As pointed out by the reviewer we indeed do not completely exclude that the anti-apoptotic effect is partially related to reduced number of pachytene cells. However, based on the fact that there is no further reduction in mitotic and meiotic cells after radiation and that normalized values still show an apoptotic reduction, we believe induction of protective mechanisms also play a role (and most likely a major role) in the observed anti-apoptotic effect.

On page 6, it is written 'upon *frh-1* and *isp-1* RNAi the number of apoptotic cells did not increase at any observed time point neither under physiological conditions nor in response to DNA-damage (Figure 2D-E).' However, Figure 2D- E clearly shows an increase in the number of apoptotic cells in time in both *isp-1* and *frh-1* knockdown animals. This is suggestive of delayed apoptotic response, possibly also because proliferation and progression through meiotic stages is slower in these animals. The authors should address this issue.

As suggested, the possibility of a delayed apoptotic response cannot be completely ruled out. However a delayed apoptotic response would result in a higher number of corpses accumulating in the time course experiments, while the observed increase in apoptotic corpses in figure 2D is not significant and therefore suggests a possible apoptotic prevention rather than a delay.

Also, the authors should clarify whether in *frh-1* and *isp-1* knockdown animals more than 12 h after the high dose of 1200 J/m² of UV-B irradiation there is still any progression of cells through meiosis at all? Could it be that meiotic maturation or progression is arrested and therefore apoptosis cannot take place anymore?

As pointed out by the reviewer, we also considered this possibility. However, it seems to be an unlikely circumstance given that during lifespan analyses upon radiations, *frh-1* and *isp-1* depleted animals, similar to control animals, were still laying eggs a few days after radiation.

Minor comments:

On page 6, the authors refer to Figure S2E when writing that in *ced-9* loss of function mutants with knockdown of *isp-1* and *frh-1* there is rescued apoptosis also after DNA-damage. This is however not shown in the figure. Please clarify.

We have now included the data also after DNA damage.

The authors should speculate why there is less PARP1 and an increase in RPA foci formation in response to ionizing radiation when *frh-1* or *isp-1* are knocked down, which is now not discussed and therefore unclear. Could the lack of PARP1 staining and increased ionizing radiation induced RPA foci indicate that (against the main conclusion) DNA repair is not functioning properly or that

there is, somehow, persistent replication stress (and/or unrepaired DNA damage) in *frh-1* and *isp-1* depleted animals?

Sure, we do not exclude the presence of replication stress and include this critical point in different parts of the revised manuscript. In the discussion section it was already specified (now underlined by referring to the relevant Figure 4) that reduced PARP recruitment could be ascribed to NAD⁺/NADH imbalance upon MRC dysfunction. When coupled with *frh-1* and *isp-1* partial resistance to genotoxic stress-induced embryonic lethality (as opposed to DDR mutants), PARP reduction and increased RPA and RAD51 foci (new data included in Figure 4) actually would argue in favor of induction of DDR (or slow resolution) to transient or low levels of DNA damage or replication stress. We have clearly stated this possibility in the discussion (Page 12): “*Although in a different study we could not observe any obvious sign of DNA damage in response to mild mitochondrial stress (Borrer et al. submitted), low levels of undetectable DNA damage or replication stress could be caused by transient increase in reactive oxygen species (ROS) production during the period of germline expansion and/or as a consequence of reduced PARP1 recruitment (Figure 4) due to NAD⁺/NADH ratio imbalance [28, 47].*”

It would help clarity of the manuscript if the authors more clearly explain their rationale for testing *gst-4::gfp* expression, as well as viability, development and fertility in their small RNAi screen described on page 8. Why are they looking for RNAi clones that increase *gst-4* expression? For instance, only half way page 8 it is explained that *gst-4* expression is used as an indirect readout for the activation of compensatory damage detoxification pathways, but this should be explained earlier to make better sense. Possibly, such 'compensatory damage detoxification pathways' and GST-4 function could be explained as part of the introduction.

To clarify the rationale of our screening we now rephrased the introductive part of the chapter and include relevant references: “*To address this possibility we carried out a small-scale genetic screen with the *isp-1(qm150); gst-4p::gfp* strain. Indeed, we have previously shown that the induction of the glutathione-S-transferase is an indirect readout for the activation of compensatory DNA-damage detoxification systems [40] and that Mit RNAi increases its expression, which is further induced by the lack of *cep-1* (the *C. elegans* p53 homolog) that concurrently suppresses *isp-1(qm150)* and Mit RNAi longevity [7, 17, 41]”.*

Table S1. It is unclear what is shown in this table. Which RNAi clones lead to increased *gst-4* expression in *isp-1* animals (only)? And where are the results for the other phenotypes scored? For instance, at the end of page 9, genes are mentioned that affect *isp-1* mutant fertility, but it is unclear where these genes are derived from. This table should be adjusted and/or better explained.

Thanks for pointing this out. We have now included a new **Appendix Table S2**, which summarize the phenotypes observed in the clones which consistently and selectively affected *isp-1;gst-4::gfp*. We also changed the genes mentioned to more closely reflect most relevant screening results.

Page 8. It is unclear how the authors go from their screening results (Table S1) to *brc-1* and *brd-1*. From which exact data in the screening results is it clear that the focus should be on these two genes?

The screening results gave us different genes, which would have been interesting to test for different reasons. We have now explained why we decided to focus on *brd/brc*, leaving other genes for future studies.

From the experiments shown in Fig 5C and D, the authors conclude that loss of *brc-1* or *brd-1* restores the ability of *frh-1* and *isp-1* depleted animals to induce apoptosis after ionizing radiation. However, an increase in ionizing radiation induced apoptosis is also observed in WT animals (contrasting results shown in Figure 2A). To be able properly compare these results, these experiments should be performed simultaneously and shown in the same graph.

Sorry for the confusion. The experiments shown in Figure 5C and D (new Figure 6) have been indeed performed at the same time but we decided to show them separately to keep the focus on the

effect of *brc/brd* on *isp-1* and *frh-1* apoptosis. As suggested we have now unified them in one graph (shown in Figure EV2D).

As pointed out by the reviewer there is some apoptosis induction in WT strain upon IR also in *isp-1* and *frh-1* RNAi animals (which is not surprising as we have noticed variations between experiments most likely reflecting differences in RNAi and/or radiation effects). Nonetheless, the number of corpses is significantly lower than irradiated control animals, and *brc-1* and *brd-1* mutants significantly suppress this anti-apoptotic effect.

To more closely reflect the figure data, we changed the sentence into “.....*lack of brc-1 or brd-1 significantly increased (rather than restore) the sensitivity of frh-1 and isp-1 RNAi animals to radiation-induced germ cell apoptosis*”.

Referee #3:

Summary

In this manuscript, the authors seek to explore the impact of mitochondrial stress-induced longevity on other aspects of cellular biology. Specifically, they investigate if mild stress on the mitochondrial respiratory chain (MRC) impacts cellular resistance to genotoxic stress. They demonstrate that MRC stress can induced germline resistance to genotoxic stress via reduced apoptosis and DNA-damage induced arrest. Using mit mutants in an RNAi screen, they were able to identify *brc-1* and *brd-1* (the BRCA1 and BARD1 homologs) as critical to these protective effects. Finally, the authors demonstrate that these genes, while important for enhanced genomic protection in the stressed MRC context, are dispensable for the enhanced longevity in these mutants. These, along with additional data presented, indicate that the DNA damage response (DDR) is uncoupled from the processes involved in longevity induced by MRC stress.

Review

This manuscript is quite well-written and clearly addresses its central narrative. The experiments used provide several lines of evidence for each point the authors present. The use of both *frh-1* and *isp-1* throughout the majority of experiments and the high agreeance of both in their behavior provide a strong argument for the findings regarding MRC stress's impact on germline DNA maintenance and genotoxic stress resistance.

There are some minor grammatical errors that should be dealt with, as well as a few concerns that are listed below. However, these issues should be easy to resolve and once addressed, will make this manuscript an even stronger report. Overall, this manuscript provides an elegant insight into how MRC stress translates to other physiological stress resistances and longevity, while simultaneously teasing about these outcomes into separate pathways. Once these issues are addressed, this report will make a significant addition to the body of work that seeks to explain the role of mitochondrial stress in longevity, which has been a hot topic of debate in the aging field for several decades.

Points to Address

1.) In Fig 2B, only data for *frh-1* is presented. The authors should include *isp-1* data for robustness or address its absence.

As suggested, we have now included *isp-1* in Figure2B in response to UVC and also in Figure 2D in response to cisplatin.

2.) In Fig 2A, it is shown that the dose of 125 Gy does not impact the RNAi fed animals. A third, higher dose should also be included to demonstrate that the system can still respond to critical amounts of damage and is not desensitized. This will also strengthen the core assertion that MRC stress increases a protective response and does not simply ignore the genetic insults.

Different data already indicate that *isp-1* and *frh-1* depleted animals are not desensitized but they can actually still sense and respond to genotoxic insults although to a less extent than wt:

- 1) Figure 2 and 3A,B – radiation induced apoptosis can still be induced although not significantly
- 2) Figure 3C-F – a normal p53/cep-1 response is induced upon genotoxic stress
- 3) Figure 4B – increase in radiation-induced RAD51 foci indicating they can sense the DNA damage
- 4) Figure 5C-H – the number of eggs laid and hatched is still partially reduced upon genotoxic stress

- 5) Figure 2D – cisplatin induces apoptosis in *isp-1* and *frh-1* depleted animals
- 6) Figure EV1C – high doses of UVB arrest development of both WT and *frh-1* silenced animals
- 7) Figure EV1 – radiation induce apoptosis still occurs in the *ced-9* and *ced-1* loss of function mutants.

These observations although clearly reveal that animals can respond differently to IR and UVB, indicate that they can in fact sense the DNA-damage but are somewhat protected.

3.) For Fig 4A, the authors state that both *frh-1* and *isp-1* gamma-induced decrease is marginal, yet *frh-1* decrease is rather significant. This needs to be addressed or discussed, as it is ~25% decrease, similar to what is observed for the control.

We have rewritten it to better reflect the results. The decrease in mitotic cells after radiation in *frh-1* animals (now in Figure 4C) is indeed significant; yet, the fold decrease upon radiation is still significantly less than in control animals.

4.) In Fig 6B, C, author's statements don't match the graphs. In Fig 6B, *frh-1* change does not appear significant. Figure legend should include statement of statistical analysis.

As summarized in Table 1 by lifespan statistics:

- 1) Figure 6B (now 7B): *frh-1* RNAi significantly extends lifespan in WT strain and also in *brc-1* mutants although to a smaller extent. Thus it is also true that the lifespan of *frh-1* RNAi on *brc-1* mutants is significantly reduced compare to its effect on the WT strain.
- 2) Figure 6C (now 7C): *frh-1* RNAi significantly extends lifespan in WT strain and even more in *brd-1* mutants.

5.) In Fig 6E, why does *glp-4* not demonstrate lifespan extension via germline ablation?

We were also surprised about this result as we expected *glp-4* mutants to live longer similar to *glp-1*. However, as far as we can tell, it has never been shown that *glp-4* loss of function actually extends lifespan.

Minor Issues

-Figure 1E should be quantified

We included quantification (Figure 1E) normalized on the number of mitotic cells.

-For Fig 5, please include qRT-PCR for *bca-1* and *brd-1*.

As suggested we have now included these new experiment indicating *brc-1* expression is increased by *frh-1*. It is also possible that the activity, rather than the expression, of these DDR genes is promoted.

-Fig 5A, no error bars for control. Please include.

These data (now in Figure 6A) are normalized on control and this is why there are no error bars displayed.

-Fig S1C, please include arrows indicating the events that were scored for this analysis.

Thanks for the suggestion. We have now replaced original panels with better pictures including arrows indicating the scored events.

2nd Editorial Decision

4 September 2018

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see, all referees are very positive about the study and request only minor changes to clarify text and figures.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

Referee #1:

The revised manuscript by Torgovnick et al., has adequately addressed the previous reviews. I ask that the authors change the Y axis on the appropriate graphs where the data is now normalized to cell numbers to indicate that it is normalized. In EV5 D and E, this is not corpses/gonad arm but PH3, CDK1 normalized for number of mitotic cells, please change. Finally, *isp-1* needs to be italicized in EV5D.

Referee #2:

I am satisfied with the author's response to my queries and think that the manuscript is now, due to its more nuanced interpretation of results, better suited for publication. The manuscript describes interesting and novel observations regarding the relation between mitochondrial dysfunction, genotoxic stress resistance and longevity, that will be of interest to the field. I therefore recommend publication.

Referee #3:

The authors have sufficiently addressed all of my concerns.

2nd Revision - authors' response

14 September 2018

The authors performed all minor editorial changes.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Natascia Ventura

Journal Submitted to: EMBO Reports

Manuscript Number: EMBOR-2018-45856V2

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if $n < 5$, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
 - are tests one-sided or two-sided?
 - are there adjustments for multiple comparisons?
 - exact statistical test results, e.g., P values = x but not P values < x;
 - definition of 'center values' as median or average;
 - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself.

Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

USEFUL LINKS FOR COMPLETING THIS FORM

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B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

| | |
|---|---|
| 1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size? | Most of the experimnts were carried out in 3 or more biological independent replicas (and in some cases each biological replica is the result of 3 technical replicas (e.g. fertility, RAD assays, qPCR). Sample number for each biological replicate has been choosen according to standard procedure in the respective fields (e.g. survival anialysis 60-100 animals per condition, apoptosis and immunostining at least 10 gonads per condition). |
| 1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used. | NA |
| 2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | NA |
| 3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe. | NA |
| For animal studies, include a statement about randomization even if no randomization was used. | NA |
| 4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe. | Yes, whenever possible we carried out experiemnts in blind. However, expert investigators can still often discriminate between wild-type or silenced animals due to the clear effect of the RNAi. We thus tried to minimize bias by haveing same experiemtns repeated by different investigators. |
| 4.b. For animal studies, include a statement about blinding even if no blinding was done | NA |
| 5. For every figure, are statistical tests justified as appropriate? | YES |
| Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. | Yes |
| Is there an estimate of variation within each group of data? | yes |
| Is the variance similar between the groups that are being statistically compared? | yes |

C- Reagents

| | |
|--|---|
| 6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right). | We included these informations in the main text (Material and methods session). |
| 7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | NA |

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

| | |
|--|--|
| 8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals. | We have included these information regarding C.elegans at the beginning of the material and methods session, |
| 9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments. | NA |
| 10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance. | NA |

E- Human Subjects

| | |
|--|----|
| 11. Identify the committee(s) approving the study protocol. | NA |
| 12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report. | NA |
| 13. For publication of patient photos, include a statement confirming that consent to publish was obtained. | NA |
| 14. Report any restrictions on the availability (and/or on the use) of human data or samples. | NA |
| 15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. | NA |
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| 17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines. | NA |

F- Data Accessibility

| | |
|---|----|
| 18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. | NA |
| Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions | |
| 19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right). | NA |
| 20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right). | NA |
| 21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biocompare (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information. | NA |

G- Dual use research of concern

| | |
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| 22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could. | NA |
|---|----|